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Review

Heterogeneity of Cancer Stem Cells: Rationale for Targeting the Stem Cell Niche

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ABSTRACT

Malignancy is fuelled by distinct subsets of stem-like cells which persist under treatment and provoke drug-resistant recurrence. Eradication of these *cancer stem cells* has therefore become a prime objective for the development and design of novel classes of anti-cancer therapeutics with improved clinical efficacy. Here, we portray potentially clinically-relevant hallmarks of cancer stem cells and focus on their recently appreciated properties of cell variability and plasticity, both of which make them elusive targets for cancer therapies. We reason that this 'disguise in heterogeneity' has fundamental implications for clinical management and elaborate on rational strategies to combat this diversity and target a broad range of tumorigenic cells. We propose exploitation of cancer stem cell niche dependence as a promising approach to interfere with various, rather than few, cancer stem cell subsets and suggest cancer-associated fibroblasts as a prime microenvironmental target for tumor stemness-depleting intervention.

Contents

Abbreviations: ABC, ATP-binding cassette; ALDH, aldehyde dehydrogenase; CAF, cancer-associated fibroblast; CSC, cancer stem cell; EMT, epithelial-to-mesenchymal transition; FACS, fluorescence-activated cell sorting; LSC, leukemic stem cell; mAb, monoclonal antibody; MGMT, O(6)-methylguanine-DNA-methyltransferase; MRD, minimal residual disease; SP, side population; TKI, tyrosine kinase inhibitor; TME, tumor microenvironment; TSC, tissue stem cell.

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1. Introduction

The perception on how tumors develop and are propagated *in vivo* has changed dramatically over the past decade. In particular, the original clonal models of cancer evolution have been abandoned and tumors are now appreciated to be tremendously complex comprising genetic and epigenetic heterogeneity within single site lesions. Moreover, comparative investigations of primary- *versus* secondary site tumor beds have revealed strong subclonal diversification of clinical metastases that might at least in part be responsible for the failure of many systemic therapies to control or eradicate metastatic disease.

One aspect of intratumoral heterogeneity is reflected by the pyramid-like structure of tumors with functionally-defined cancer stem cells (CSCs) at the apex of the malignant hierarchy. Conserved in most tumor entities, CSCs, or cancer-initiating cells, are endowed with unique functional properties and dictate the whole course of tumor evolution including cancer initiation, metastatic progression, and disease recurrence after clinical remission. Thus, these cells have emerged as a highly attractive target population for anti-cancer treatment, and strategies to eliminate these cells are being heavily explored. However, recent evidence has suggested that aside from dormancy and detoxification, CSC targeting approaches are faced with additional challenges including low immunogenicity of CSCs, cellular heterogeneity of CSC pools, and a general plasticity of stemness phenotypes. In this review, we summarize the latest advances in our understanding of CSC biology and function, and highlight potential implications of tumor cell variability for the conceptual design of CSC-directed therapies. We propose CSC heterogeneity as yet another example for Darwinian selection during tumor progression and suggest that microenvironment-targeted strategies will guide the development of anti-CSC treatments in the future, based on the inherent niche dependence of CSC populations.

2. The Cancer Stem Cell Concept

Organ development –and homeostasis depends on small populations of dedicated stem cells, which maintain tissues by continuous replacement and also secure demand-adapted regeneration in case of emergencies, such as injury [1]. Functionally, stem cells are characterized by their selective ability for self-renewal and differentiation, which allows them to generate all cell lineages within a given tissue [1]. Furthermore, stem cells exhibit a high degree of evolutionary fitness conferred, amongst others, by sophisticated mechanisms of detoxification [2,3] and residence in protective microenvironments (i.e., stem cell niches) [4,5].

Starting with the seminal article of Al-Hajj and co-workers in 2003 [6], the principles of stem cell biology have been increasingly used to explain basic biological and clinico-pathological features of cancer, even though the first connection between stem cells and malignancies were already proposed in the mid-20th century [7,8]. In particular, it is now appreciated that cancer arises from the malignant transformation of a stem/progenitor cell or, alternatively, from a non-stem cell that has regained stemness potential by a dedifferentiation process [9–11]. This paradigm is corroborated by the remarkable convergence of stem cells and CSCs in terms of preferentially activated signalling cascades, as well as their overlapping expression of certain markers. As an example, both stem cells and CSCs show activation of the self-renewal-associated pathways Wnt/β-catenin, Bmi-1, sonic hedgehog, Notch and PTEN [12], and both populations express tissue-specific stem cell markers, such as CD34 (blood) [13,14] and Lgr5 (colon) [15,16]. Importantly, this concordant molecular profile is reflected in several key aspects of CSC biology including longevity, dormancy/quiescence, niche dependence, and the potential for asymmetric cell division [17–20]. Accordingly, CSCs are selectively required for cancer initiation and subsequent propagation, properties that have led to the designation of CSCs as the 'beating heart' of malignant growth [18], and to their declaration as prime therapeutic targets [21]. Methodologically, CSCs can be purified from biological samples using flow cytometry/FACS employing phenotypic markers such as CD44 and CD133, or functional characteristics such as dye extrusion and enzymatic activity [22]. On the functional level, *bona fide* CSCs show tumor-initiating potential *in vivo*, are capable of anchorage-independent growth *in vitro* and are notably resistant to cytotoxic and targeted anti-cancer drugs as well as radiotherapy [18–20]. However, it has to be stressed that the frequency and identity as well as other hallmarks of CSCs vary substantially among tumor entities (Table 1). In addition, methodological factors such as the particular experimental conditions used can impact the detection of CSCs. As an example, tumor engraftment in more severely immune-compromised mice increases the detectable frequency of tumorigenic cells by several orders of magnitude [23], demonstrating the challenges in implementing a universal definition of CSCs.

Several landmark studies have established that the transition from single site tumor growth to life-threatening metastatic disease is mechanistically enabled by CSCs, which seem to have particular resistance to the rate-limiting steps of the metastasis cascade including anoikis, extravasation, and re-settlement/survival in 'unnatural' environments [24–26]. Accordingly, metastatic cancer cells are enriched in stemness-associated gene signatures and also show functional stem cell properties [27]. One missing link between stem cell traits and metastasis could be the recent appreciation that CSCs exhibit a distinct transcriptional program otherwise found during developmental tissue remodelling and commonly referred to as epithelial-to-mesenchymal transition (EMT) [28,29]. This could at least in part explain the increased migratory potential of these cells, as well as their poor response to treatment. Importantly, the relationship between EMT and CSCs seems to be causal, because if cells are forced to undergo an EMT (e.g., by treatment with TGF-β, knockdown of E-cadherin, or ectopic expression of the EMT transcription factors TWIST or Snail), they simultaneously acquire phenotypic and functional properties of stem cells [30,31].

2.1. Cancer Stem Cells are Therapy-Resistant and Mediate Disease Recurrence

Clinically, the relevance of CSCs is largely seen in their intrinsic resistance to various cytotoxic and targeted anti-cancer drugs, which secures their persistence during treatment and predisposes the patient to relapse [2]. This is in line with studies showing that states of remission or minimal residual disease (MRD), which often escape clinical detection, are established and sustained specifically by CSCs [32–35]. Indeed, CSCs are selected during *in vivo* chemotherapy, and recurrent tumors are enriched in CSCs or CSC-related gene signatures [36,37]. Along similar lines, expression of surrogate CSC markers correlates with reduced survival in different tumor entities and also predicts poor response to therapeutic intervention [38–40].

Several non-overlapping mechanisms of protection contribute to the treatment refractoriness of CSCs. For instance, their inherent tendency to remain quiescent over extended periods of time substantially reduces their sensitivity to anti-proliferative drugs such as classical cytostatics